PROPERTIES OF THE DACTYL CHEMORECEPTORS OF CANCER ANTENNARIUS STIMPSON AND C. PRODUCTUS RANDALL[1]

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Behavioral and electrophysiological observations have indicated the presence of chemoreceptors particularly sensitive to amino acids on the dactylopodites of the green crab, *Carcinus* (= *Carcinides*) *maenas* (Case, Gwilliam and Hanson, 1960; Case and Gwilliam, 1961). Although sufficient information regarding chemoreception in various arthropods previously had accumulated to render those observations not unexpected (Luther, 1930; Hodgson, 1958; Barber, 1961), recently Laverack (1963) has been unable to detect responses to amino acids in an electrophysiological examination of the dactyl innervation of the European *Carcinus*. Here we attempt to resolve the problems thus raised concerning the reality of dactyl amino acid receptors in crabs by demonstrating their presence in two species of yet another brachyuran genus and by considering their chemical sensitivity in some detail.

Preliminary reports of this investigation have appeared (Case and Gwilliam, 1963; Case, 1964).

Materials and Methods

The experimental material consisted primarily of dactylopodites of any pereiopod of mature *Cancer productus* Randall and *C. antennarius* Stimpson. No variations in sensory responses could be attributed to differences between species or sexes or among legs. Dactyls were prepared for recording, after limb transsection between propodite and carpopodite, by dissection of musculature and articulations of the dactylopodite-propodite joint, leaving undisturbed the centrally placed nerve. The distal centimeter of the dactyl was then pushed through a small hole in a rubber sheet which formed one end of a sea water-filled chamber into which the nerve floated as the propodite was pulled away. Nerve bundles were subdivided and arranged for monopolar recording at the water surface on a bare silver electrode leading to a P5C Grass A.C. amplifier. Amplified signals were led to an audio monitor and Tektronix 502 oscilloscope. Provisions were available for tape recording and for signal integration, the latter by means of a modified Offner myograph integrator (1.0 sec. time constant) and oscillograph.

Test materials were made up in sea water, neutralized except where specified, and applied usually as approximately 0.03-ml. single drops to the moist dactyl tip in air. Washing with at least 15 drops of sea water followed the stimulus drop within a few seconds except when persistent stimulant effects were under study. Test and wash solutions and the preparations were all held at 16° to 18° C.

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Study of the receptors of any dactyl began with selection of a subdivided bundle containing only a few chemoreceptive units, all giving substantial responses to single drops of 0.05 *M* glycine and with endings suitably placed for stimulation in a restricted area on the dactyl tip proximal to the heavily chitinized cap. Response magnitude was ordinarily determined from the first 1.5 second of the integrated response and expressed as an activity ratio (AR), the ratio of the experimental response to the reaction of that same preparation to 0.05 *M* glycine. Since the standard glycine response was determined every few tests, expressing stimulatory effectiveness in this way served to compensate for temporal variation in sensitivity on the part of the same preparation, for variation in sensitivity and number of active units among preparations, and for the contribution of non-chemoreceptive units to the response.

Results and Specific Comments

1. Discrimination between mechano- and chemoreceptors. Mechanoreceptor activity commonly occurs along with chemoreceptor responses within subdivided nerve bundles, as in Figure 1A, although not necessarily (Fig. 5). Continued subdivision of bundles carrying mixed activity frequently results in isolation of fascicles in which chemoreceptor units are dominant, the situation in Figure 1B. Note, however, that this investigation offers no conclusive evidence concerning the possibility that chemoreceptor units may also be excited by physiologically reason-

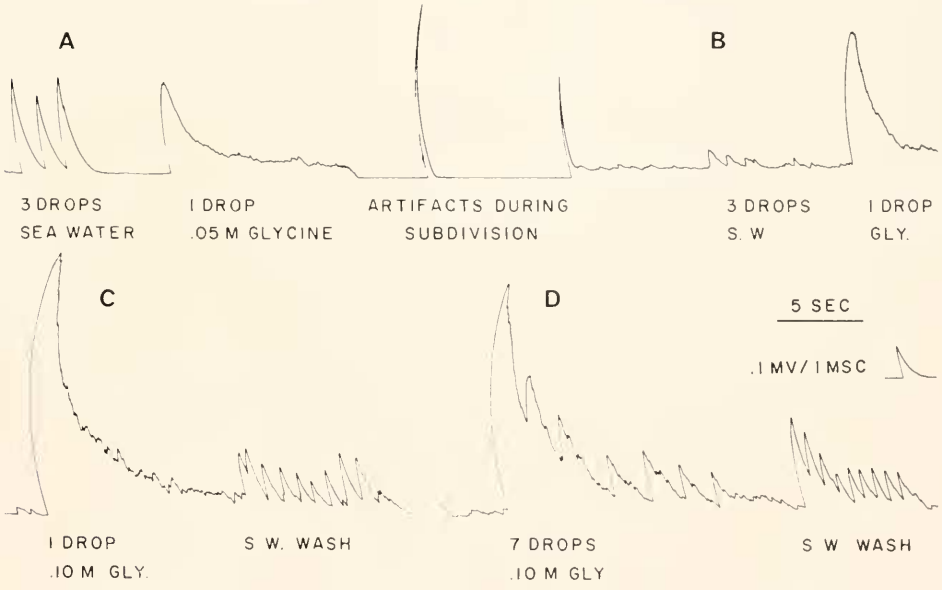


Figure 1. Integrated responses from dactyl innervation. A and B demonstrate improvement in recording of chemoreceptor activity in course of subdivision of nerve. C and D, records from another dactyl showing similar rates of response decay when dactyl is stimulated with either one or several drops of stimulant. Notches in D indicate mechanoreceptive responses to successive drops of test solution. *C. productus.*

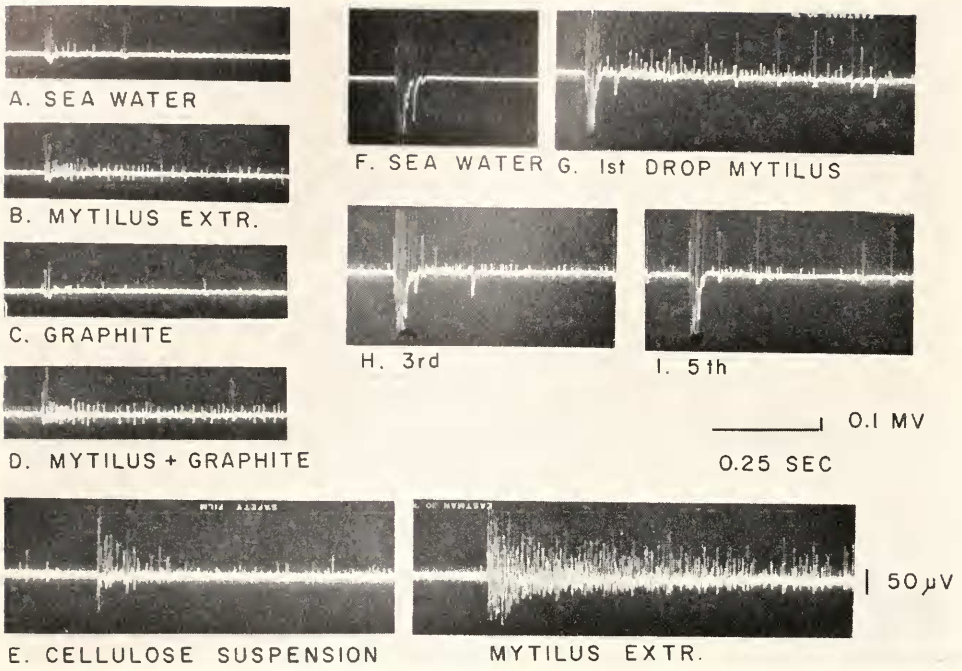


FIGURE 2. Comparison of responses to mechanical and chemical stimulation of dactyl. See text for details. *C. productus.*

able mechanical stimuli. That a form of synergy may occur between chemical and mechanical stimuli is frequently suggested by situations in which the first drops of sea water wash after chemical stimulation elicit considerably larger responses than later drops in the wash series, as illustrated in the wash responses of Figure 1D.

When both kinds of sensory activity appear in the same fascicle, they are readily distinguished by simple experiments of the type demonstrated in Figure 2, in this instance specifically concerned with the extent to which a sea water extract of *Mytilus* tissue stimulates chemically and mechanically. Three different fascicles are represented, all obviously containing mechanically sensitive components as shown in Figure 2A, E, F. The responses in Figure 2A, B are obviously unlike in that the extract causes prolonged activity in small units which are not activated by the sea water control. Since a microscopic examination shows that filtrate is by no means particle-free, a thick suspension of graphite[2] particles, similar in size to those found in the extract, is applied, with the minimal effect apparent in Figure 2C. Subsequent application of the extract without washing away the graphite shows that the previous treatment has not inactivated the receptors (Fig. 2D). The even larger particles of a cellulose suspension produce only minimal responses as compared with the extract in a test on another fascicle (Fig. 2E, H).

[2] "Prodag," Acheson Colloids Co. semi-colloidal graphite, used after prolonged washing.

TABLE I

Summary of dactyl preparations responsive to 0.05 M glycine

	Responsive	Non-responsive	Per cent responsive
C. productus (n = 28)			
2d pereiopod	15	6	
3d pereiopod	9	4	
4th pereiopod	11	2	
5th pereiopod	11	5	
Unspecified	10	0	
	56	17	77 (73 preparations)
C. antennarius (n = 30)			
2d pereiopod	34	5	
3d pereiopod	26	3	
4th pereiopod	25	1	
5th pereiopod	14	1	
	99	10	91 (109 preparations)
Totals, both species	155	27	85 (182 preparations)

Another means of differentiating between mechano- and chemoreceptor units is illustrated in the remainder of Figure 2. A pure mechanoreceptive response to a drop of sea water occurs in Figure 2F. The remaining illustrations in the series, Figure 2G, H, I, are examples of responses to a long series of drops of *Mytilus* extract. It is clear that the chemoreceptor element of the response

TABLE II

Examples of distribution of chemo- (CR) and mechanoreceptor (MR) activity in dactylus and propus innervation

Fascicle (in order of testing)	*C. productus* 5th. pereiopod				*C. antennarius* 4th. pereiopod			
	Dactylus		Propus		Dactylus		Propus	
	CR	MR	CR	MR	CR	MR	CR	MR
1	+	+	0	0	0	0	+	+
2	+	+	+	+	0	0	0	0
3	0	0	0	0	0	0	0	+
4	+	+	0	+	0	0	0	+
5	+	+	0	0	+	0	0	+
6	0	+	0	0	0	0	0	+
7	+	+	0	0	+	+	+	+
8	0	0	0	0	+	+	0	+
9	0	0	0	0	+	+	+	0
10	0	+	0	+	+	+	0	0
11	+	+	0	0	+	+	0	0
Total +	6	8	1	3	6	5	3	7

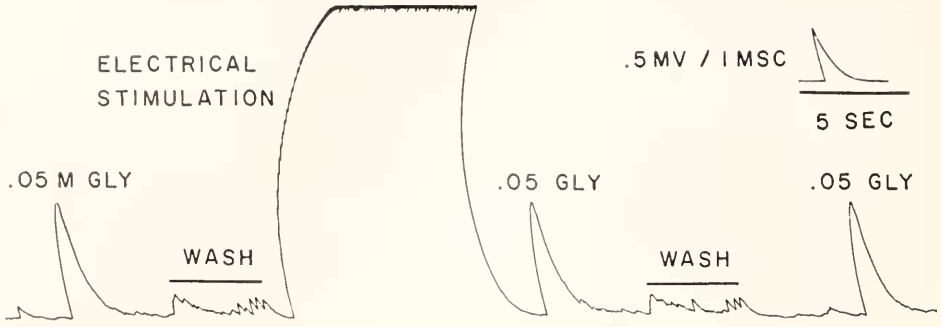


FIGURE 3. Demonstration that decay of chemoreceptor response is not due to fatigue of electrically excitable receptor elements. See text for details. *C. antennarius.*

adapts considerably more readily than the mechanoreceptive element. This aspect of chemoreceptor activity is discussed in more detail below.

2. Prevalence and distribution of chemosensory units. The percentage of preparations responsive to 0.05 *M* glycine in the entire experimental series is presented in Table I, from which it is evident that amino acid-sensitive units are common on all pereiopods of both *C. productus* and *C. antennarius.* The frequency of successful preparations is probably unrealistically low in the case of *C. productus* since most failures to demonstrate chemoreceptors in that species involved only five crabs in which chemoreceptors were not evident in any limb.

Few data regarding chemosensitivity of chelipeds have been obtained because their size makes them awkward to prepare for recording. It can only be reported that both of two chelipeds of *C. antennarius* which were tested were responsive to 0.05 *M* glycine, the only substance tested. Chelipeds have been shown behaviorally to mediate feeding responses to several amino acids (Case and Gwilliam, 1961; 1963).

Undoubtedly chemoreceptors are not confined to the dactylopodites. This

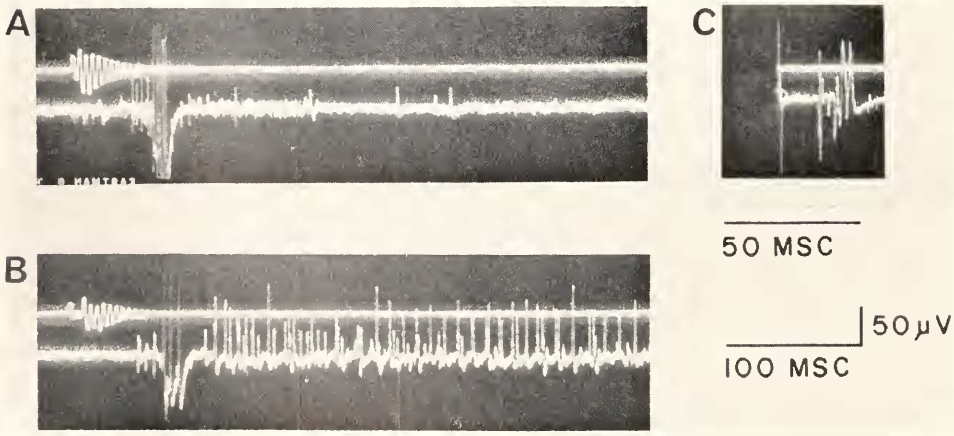


FIGURE 4. Illustration of method of determination of latency; 100-msec. calibration applies to A and B. See text for details. *C. antennarius.*

is demonstrated in Table II which summarizes the distribution of chemo- and mechanoreceptor activity in a number of randomly selected fascicles from the innervation of both dactylopodite and propodite. Obviously both limb segments are well supplied with chemoreceptor endings. No significance can be attached to the indication of a larger number of chemoreceptive units in the dactyl, since the experimental arrangement permitted exploration of little more than half the propodite surface, while nearly all the dactyl surface was exposed to testing.

Although more proximal limb segments have not been tested electrophysiologically, behavioral tests show that they do mediate feeding responses, as contrasted, for example, with the dorsal body surface (Case, unpublished observations).

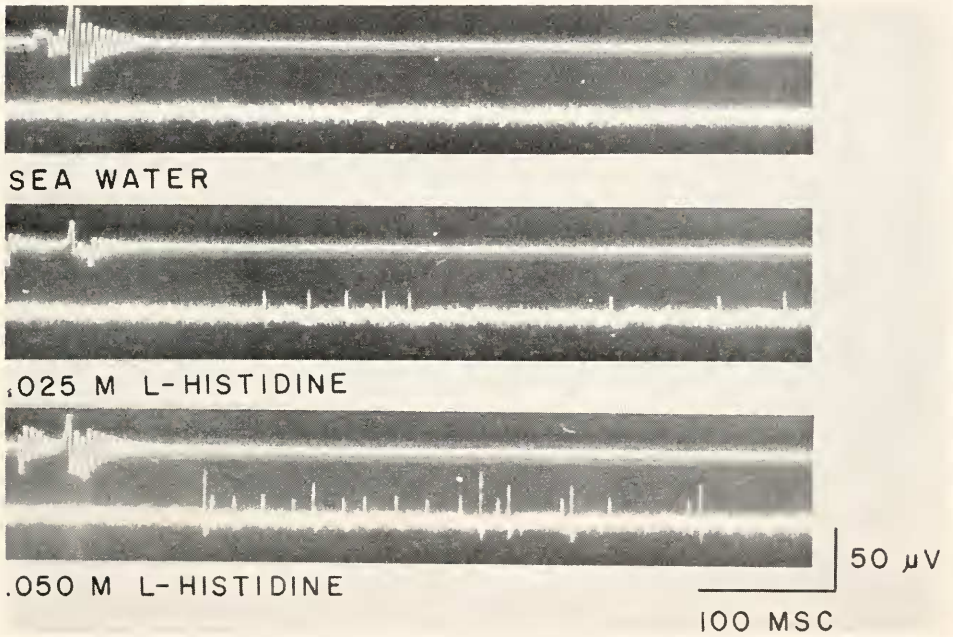


FIGURE 5. Responses of a pure chemoreceptor bundle to 0.05 *M* L-histidine, showing effect of concentration on threshold and latency of two units. Widest excursion of upper trace indicates arrival of stimulus drop. *C. antennarius.*

3. Temporal characteristics of chemoreceptor responses. The usual time course of responses to effective chemical stimulants consists of attainment of maximum activity within the first 0.2 second after stimulus application with a markedly slower decline to half maximum in one to two seconds. Activity subsequently approaches the resting level more slowly with no obvious suggestion of stabilizing above rest level. As far as approximately the initial 10 seconds of the response are concerned, this pattern, well illustrated in the integrated records of Figure 1A, B, cannot be the consequence of the technique of stimulation, namely application of single drops of test solutions. Thus, in Figure 1D the response curve in which seven drops of glycine are applied is virtually identical with the response

in Figure 1C, produced by a single drop of glycine, save for mechanoreceptor responses.

Conceivably, rapid response decay can be attributed to effects upon specific chemoexcitatory processes leading to impulse generation. When, as in Figure 3, a small receptor population is excited electrically with an electrode pair placed nearby on the integument, all peripheral, electrically excitable elements of the impulse-generating sequence, except the specific chemosensory mechanism, are undoubtedly excited. Activation of the receptors in this way, to an extent far

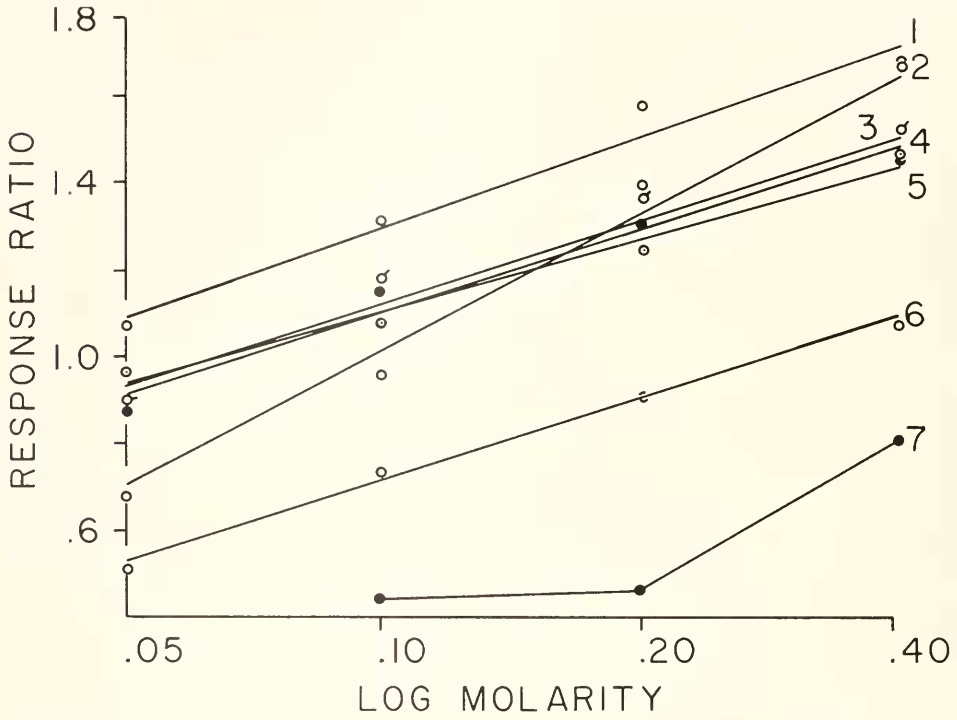


FIGURE 6. Concentration-activity relationships of various stimulants. 1, L-serine; 2, L-alanine; 3, glycine; 4, L-proline; 5, L-threonine; 6, glycyl-glycine; 7, trimethylamine. Plots 1–6 calculated by least squares. Five preparations tested at all concentrations of all compounds in ascending order of concentrations with two-minute washes between tests. Data not used in the tabulations of this paper. *C. antennarius*.

greater than the response to a standard glycine stimulus, has no evident effect upon an immediately ensuing response to chemical stimulation.

Somewhat different responses were observed more rarely. These were characterized by abrupt transition from a decay curve of the usual form to a much more gradual decline towards rest level. If fascicles behaving in this manner did arrive at an elevated steady-state, rest level was probably not exceeded by more than 10%. We have no clear idea at present concerning the significance of such responses. Their rarity and the fact that they resemble the obviously abnormal responses to high concentrations of certain chemical agents (see below) suggests

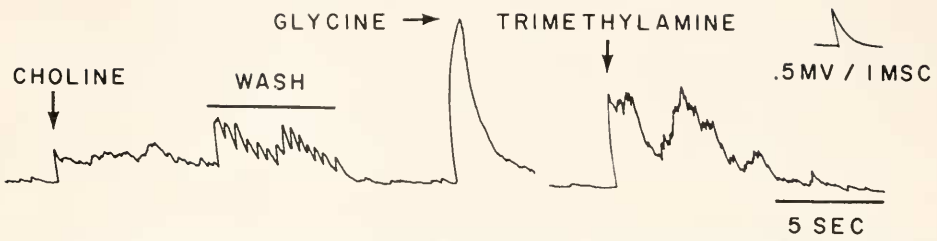


FIGURE 7. Comparison of responses to 0.40 *M* choline, 0.05 *M* glycine and 0.40 *M* trimethylamine. Approximately 5 seconds deleted from record between glycine and trimethylamine.

they are not typical. Consequently, preparations responding in this manner to 0.05 *M* glycine have not been used further in this investigation.

Response latency was determined approximately by means of a phonograph pick-up with a fine glass stylus positioned immediately above the dactyl in the path of the drop of stimulant, as illustrated in Figure 4, where response to a drop of sea water (Fig. 4A) appears in contrast with the response of the same units to 0.01 *M* L-glutamic acid (Fig. 4B). In each display initial deflection of the upper trace signals contact of the stimulus drop with the stylus, with the largest deflection when the drop leaves the stylus. The earliest mechanoreceptor activity in Figure 4A appears after 24 msec. while the earliest obvious chemoreceptor activity is seen after about 56 msec. in Figure 4B. Conduction velocities of 2.4 meters/sec. for chemoreceptor and 3.5 meters/sec. for mechanoreceptor axons were estimated for this preparation by measuring the rate of propagation over a 21-mm. length of the same bundle (Fig. 4C). The latencies for this preparation consequently become 9.8 msec. for the earliest mechanoreceptor and 35 msec. for the earliest chemoreceptor response to glutamic acid. Chemoreceptor latencies can obviously be

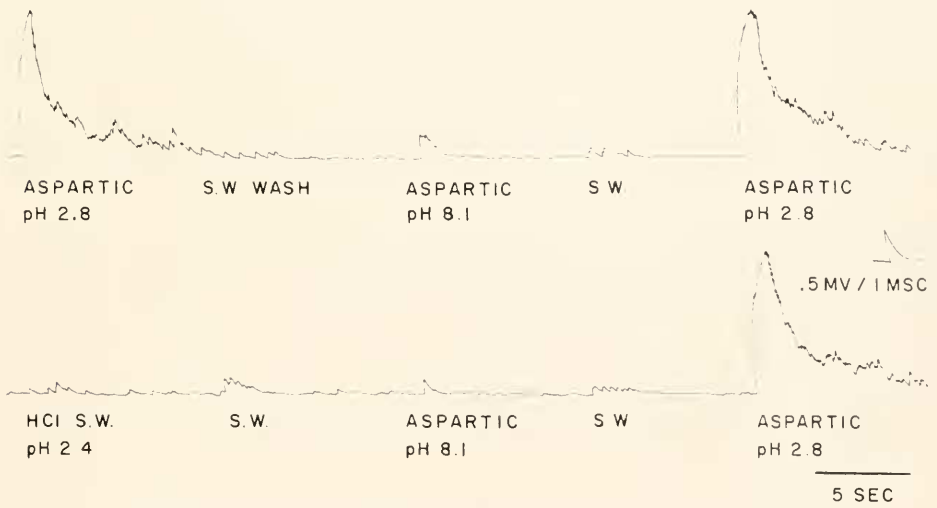


FIGURE 8. Influence of pH on response to 0.04 *M* D-aspartic acid. Upper record continuous with lower. *C. productus.*

expected to vary with the nature of the stimulant and with stimulant concentration, as illustrated in Figure 5 where the smallest of the three chemoreceptors responds earlier to a higher concentration of histidine.

4. Response magnitude as a function of stimulant concentration. Response magnitude of effective stimulants is linearly related to the logarithm of the concentration (Fig. 6). Six compounds, representative of the array of substances which appear to be effective stimulants, were tested in the range 0.05 to 0.04 *M*

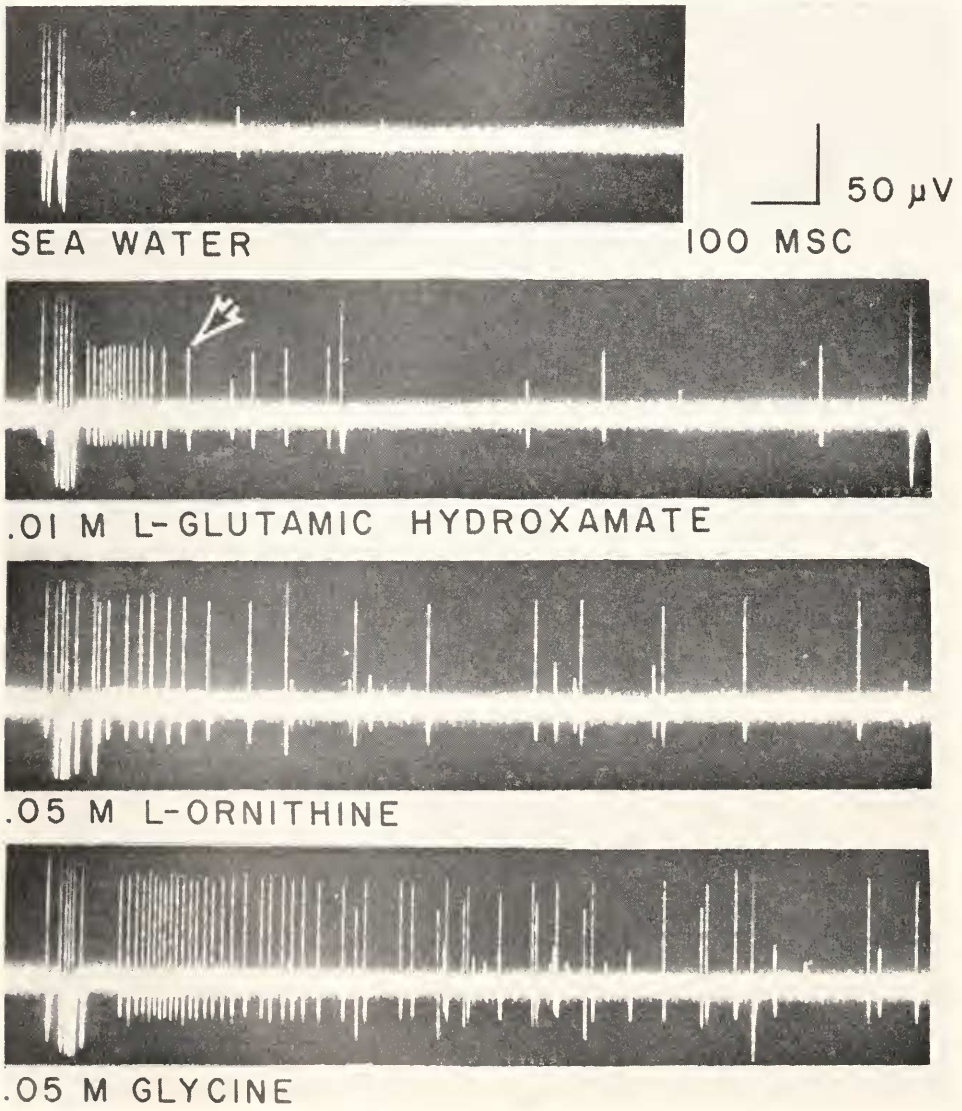


FIGURE 9. Multifiber preparation illustrating variation in chemical specificity. See text. *C. antennarius.*

TABLE III

Activity ratios of amino acids and related compounds. I. Aliphatic amino acids

Compound	Structure	AR*	SD**	N***
glycine	$CH_2(NH_2)CO_2H$	1.00 (arbitrary)		
N-carbamyl glycine	$(NH_2)CONHCH_2CO_2H$	0.25	± 0.10	13
N,N-dimethyl glycine	$(CH_3)_2NCH_2CO_2H$	0.75	0.21	11
N-methyl glycine (sarcosine)	$CH_3NHCH_2CO_2H$	0.98	0.17	10
L-alanine	$CH_3CH(NH_2)CO_2H$	0.88	0.16	10
D-alanine	—	0.70	0.14	10
β-alanine	$NH_2CH_2CH_2CO_2H$	0.81	0.11	9
L-serine	$CH_2(OH)CH(NH_2)CO_2H$	1.13	0.23	9
taurine	$HO_3SCH_2CH_2(NH_2)$	1.41	0.26	10
cysteine	$CH_2SHCH(NH_2)CO_2H$	0.79	—	5
DL-α-amino-n-butyric acid	$CH_3CH_2CH(NH_2)CO_2H$	1.70	0.37	10
α-amino isobutyric acid	$(CH_3)_2C(NH_2)CO_2H$	0.38	0.14	9
DL-β-amino isobutyric acid	$(NH_2)CH_2CH(CH_3)CO_2H$	1.12	0.10	8
DL-β-amino butyric acid	$CH_3CH(NH_2)CH_2CO_2H$	0.92	0.06	9
γ-amino butyric acid	$NH_2CH_2(CH_2)_2CO_2H$	0.31	0.12	11
L-threonine	$CH_3CH(OH)CH(NH_2)CO_2H$	1.05	0.17	11
L-methionine	$CH_3SCHCH_2CH(NH_2)CO_2H$	0.78	0.13	10
α-methyl-DL-methionine	$CH_3SCHCH_2C(CH_3)(NH_2)CO_2H$	0.68	0.11	7
L-valine	$(CH_3)_2CHCH(NH_2)CO_2H$	1.00	0.19	9
D-valine	—	1.00	0.17	8
L-leucine	$(CH_3)_2CHCH_2CH(NH_2)CO_2H$	0.78	0.18	8
D-leucine	—	0.56	0.12	11
L-norleucine	$CH_3(CH_2)_3CHNH_2CO_2H$	0.61	0.11	9
L-isoleucine	$CH_3CH_2CH(CH_3)CH(NH_2)CO_2H$	0.73	0.21	11
D-isoleucine	—	0.54	0.15	9
L-lysine	$CH_2(NH_2)(CH_2)_3CH(NH_2)CO_2H$	0.37	0.08	10
ε-amino caproic acid	$(NH_2)CH_2(CH_2)_4CO_2H$	0.27	0.07	7
L-arginine	$(NH_2)C(NH)NHCH_2(CH_2)_3CH(NH_2)CO_2H$	0.39	0.09	11
α-ε-diaminopimelic acid	$HO_2CC(NH_2)(CH_2)_3CH(NH_2)CO_2H$	0.29	0.17	13

* Activity ratio; ** standard deviation; *** number of tests. Number of animals tested is approximately ½ this.

on *C. antennarius*. Of these, only L-alanine significantly differed in regression from the others ($P < 0.01$) while all six regressions of concentration *versus* response were significantly linear. Some compounds, such as trimethylamine and choline, do not appear to have a linear dose-response relationship in the same concentration range. As indicated in Figure 6, trimethylamine is essentially inactive until a concentration somewhat above 0.10 *M* is attained, whereupon response magnitude increases rapidly. An integrated record of response to 0.40 *M* trimethylamine is illustrated in Figure 7 in comparison with a standard glycine response. Trimethylamine responses are typically as shown, often bimodal and markedly different from the typical glycine response pattern. Choline, like trimethylamine, is ineffective except at high concentrations which produce a sustained, low intensity response.

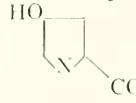
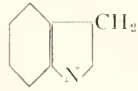
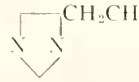
5. The effect of pH. The receptors are not excited either by acids such as hydrochloric or acetic at pH 2.0 or by sodium hydroxide at pH 9.0. Glycine and proline underwent no change in excitation efficiency over the same pH range.

In contrast to these observations L-aspartic acid is at least 10 times as active in acid solutions as near neutrality (Fig. 8). The speculation engendered by this observation, that the undissociated molecule might be the more excitatory, was not supported in the present experiments by the uniform activity of L-arginine in both alkaline and neutral solutions.

6. The adequate stimulus of the dactyl receptors. The observations of this section were obtained from *C. antennarius.*

TABLE IV

Activity ratios of amino acids and related compounds. II. Dicarboxylic Acids

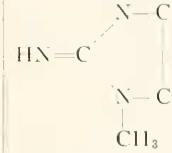
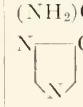
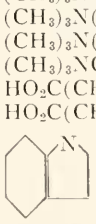
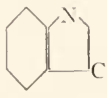
Compound	Structure	AR	SD	N
L-aspartic acid	$HO_2CCH_2CH(NH_2)CO_2H$	0.29	± 0.15	24
D-aspartic acid	—	0.93	0.13	20
DL-β-methyl aspartic acid	$HO_2CCH_2C(CH_3)(NH_2)CO_2H$	0.22	0.14	22
L-glutamic acid	$HO_2C(CH_2)_2CH(NH_2)CO_2H$	1.37	0.29	12
D-glutamic acid	—	0.51	0.19	10
N-carbamyl-L-glutamic acid	$HO_2C(CH_2)_2CH(NHCONH_2)CO_2H$	0.20	0.10	15
DL-N-methyl glutamic acid	$HO_2C(CH_2)_2CH(NHCH_3)CO_2H$	0.24	0.15	8
L-α-amino adipic acid	$HO_2C(CH_2)_3CH(NH_2)CO_2H$	0.58	0.20	7
	III. Aromatic and Heterocylic compounds			
L-phenylalanine		0.38	0.16	10
L-proline		1.00	0.17	14
hydroxy-L-proline		1.00	0.22	7
L-tryptophan		0.34	0.11	10
L-histidine		0.81	0.28	14
D-histidine		0.46	0.22	8

One aspect of the experimental method must be emphasized in regard to these experiments, namely that receptor populations have been examined, not single units. At this juncture it is impossible to determine how many receptor types, described in terms of response specificities, constitute the dactyl chemoreceptor population. While it is true that units conforming to our selection criterion of fast-adapting responses to 0.05 *M* glycine do respond in a uniform manner to many other chemical stimuli, there are certainly population variations in threshold and absolute specificity. To illustrate: D- and L-aspartic acids have never been tested without finding D-aspartic acid to be at least five times as active as its enantiomer (see below), an unexpectedly fine discrimination, but one evidently found in all

members of the receptor population; yet threshold variations are common among members of this same population, as in Figure 5 where the two larger units are evidently less sensitive to L-histidine than the smaller one. Further, evidence of absolute specificity differences is common, as exemplified in Figure 9 where one unit is responsive to a glutamic acid derivative but not to L-ornithine and probably not to glycine. We believe, however, that these variations within the receptor population are minor enough to justify a survey of chemical specificity with multiple unit preparations selected according to the criteria described.

TABLE V

Activity ratios of amino acids and related compounds. IV. Amides, Amines, Miscellaneous

Compound	Structure	AR	SD	N
L-asparagine	$CONH_2CH_2C(NH_2)CO_2H$	0.70	± 0.10	12
creatinine		0.37	0.18	16
L-glutamine	$(NH_2)OCH_2(CH_2)_2CH(NH_2)CO_2H$	0.61	0.12	7
ethanolamine	$HOCH_2CH_2NH_2$	0.41	0.08	7
cadavarine	$(NH_2)CH_2(CH_2)_3CH_2(NH_2)$	0.09	0.10	12
histamine		0.30	0.17	11
trimethylamine	$(CH_3)_3N$	0.40	0.09	12
glycine betaine	$(CH_3)_3NCH_2CO_2H$	1.06	0.10	9
choline	$(CH_3)_3N(OH)CHCH_2OH$	0.25	0.11	9
acetylcholine	$(CH_3)_3N(CH_2)_2OCOCH_3$	0.26	0.07	9
DL-carnitine	$(CH_3)_3NCH_2CH(OH)CH_2CO_2H$	0.33	0.10	13
glutaric acid	$HO_2C(CH_2)_3CO_2H$	0.35	0.18	20
α-ketoglutaric acid	$HO_2C(CH_2)_2COCO_2H$	0.25	0.19	13
indole		0.20	0.09	8
3-methyl indole		0.15	0.07	8
urea	$OC(NH_2)_2$	0.17	0.09	8

The principal results of the survey of chemical specificity are contained in Tables II, IV, V, and VI and Figure 7 with the following observations emergent:

The receptors are highly responsive to α-amino acids and certain related substances. The preponderance of evidence set forth in the tabulations shows the receptors are most responsive to α-amino and α-imino acids. Neither organic acids nor amines are conspicuously active (Tables III, IV and V). Indole, 3-methyl indole and trimethylamine, which might well appear in the dietary of these

crabs, are similarly ineffective. The sugars, sucrose, glucose and trehalose, were non-stimulatory, as were all salts tested, namely KCl, NH_4Cl and Na acetate.

The response spectrum to α-amino compounds is not restricted. The imino acids, L-proline and hydroxy-L-proline, are both as effective as glycine, for example. Nonetheless, activity is clearly affected by certain structural modifications, as follows: (i) Increasing size of the molecule reduces activity. Among the α-amino acids this is most apparent in the instance of α-ϵ-diaminopimelic acid and L-α-aminoadipic acid. Maximal activity appears to occur in straight chain amino acids at C = 3 to 5. The dipeptides tested (Table VI) are all less active than their most active constituent amino acid, except possibly in the instance of L-lysyl-L-glutamic acid. Triglycine and tetraglycine exhibit progressively diminishing activities and polyglutamic acid and the two proteins studied were inactive.

TABLE VI

Activity ratios of peptides and proteins

Compound	Typical active constituent	AR	SD	N	t*
glycyl-glycine		0.66	0.12	25	
triglycine		0.50	0.09	15	
tetraglyciae		0.40	0.10	12	—
	glycine	1.00 (arbitrary)			
glycyl-L-proline		0.36	0.10	10	0.001
	L-proline	1.00	0.17	14	
glycyl-DL-sarcosine		0.41	0.17	8	0.01
	DL-sarcosine	0.98	0.17	10	
glycyl-L-glutamic acid		0.41	0.11	10	0.001
	L-glutamic acid	1.37	0.29	12	
L-lysyl-L-glutamic acid		1.00	0.33	10	nsd
	L-glutamic acid	1.37	0.29	12	
polyglutamic acid (MW = 40,000–100,000) 25 mg./ml.		less than 0.1		5	
albumin, egg, 5 × cryst. 25 mg./ml.		less than 0.1		5	
haemoglobin, bovine, 2 × cryst. 25 mg./ml.		less than 0.1		5	

* Student's "t" test.

The inactivity of these last three substances might possibly be simply related to concentration. (ii) Only configurations approximating α-amino are effective. Consider in this regard the aminobutyric acids. DL-α-amino-n-butyric acid is the most active compound yet tested while γ-amino butyric acid is among the least active. α-amino isobutyric acid is also inactive, perhaps due to steric hindrance introduced by methyl substitution on the α-carbon. β-amino isobutyric acid, still quite active, would appear to be an exception to this proposition, except for the fact that this molecule can assume a configuration in which the carboxyl and β-amino groups are in the same steric relationship as in an α-amino compound. A similar argument is possible regarding β-alanine.

Certain other substituted compounds indicate the importance of the α-amino configuration to excitation. These include N-carbamyl and N-methyl substitutions, especially of glutamic acid, which reduce the AR from 1.37 to 0.20 and 0.24,

respectively. Similarly, N-carbamyl glycine is quite inactive although N,N-dimethyl glycine appears to be an exception, as well as α-methyl DL-methionine. In the last instance the slight decrease in activity, if significant, might be due either to the α-methyl substituent or perhaps to D-methionine, whose activity has not been determined but which may well be less than L-methionine. Finally,

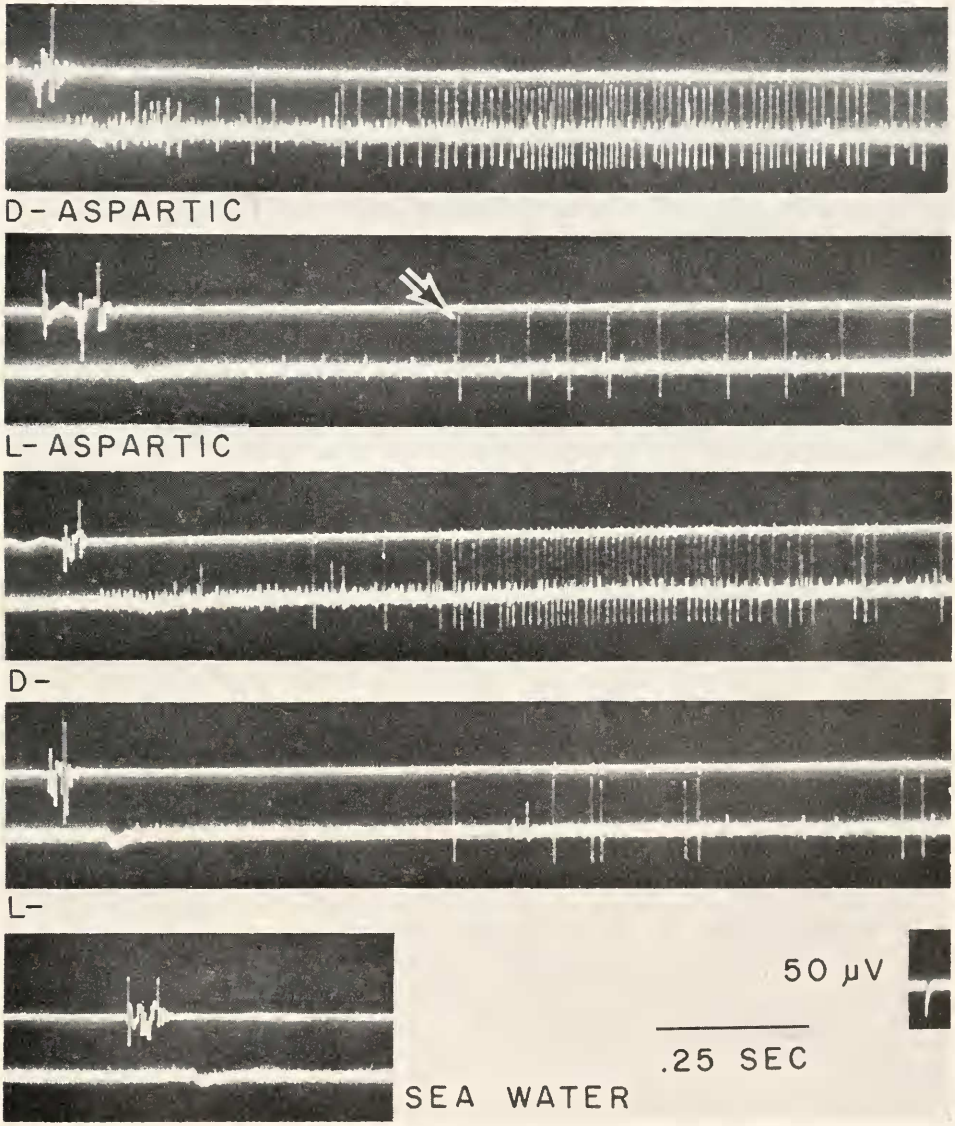


FIGURE 10. Demonstration of differential response of unit marked with arrow to D- and L-aspartic acid, 0.04 *M*. Records were taken sequentially with 5-second wash between each. *C. antennarius*.

even β- substitution may significantly impair activity, as in the instance of DL-β-methyl aspartic acid, although this effect is not realized in isoleucine which actually is as active as leucine and nor-leucine.

The data of Tables III, IV, and V demonstrate that members of several sterioisomer pairs do not have equal activities. D- is markedly more active than L-aspartic acid, while L- isomers of glutamic acid, leucine, iso-leucine and histidine are more active than their enantiomorphs. On the other hand, both isomers of alanine and valine are probably equally effective stimulants. At least in the instance

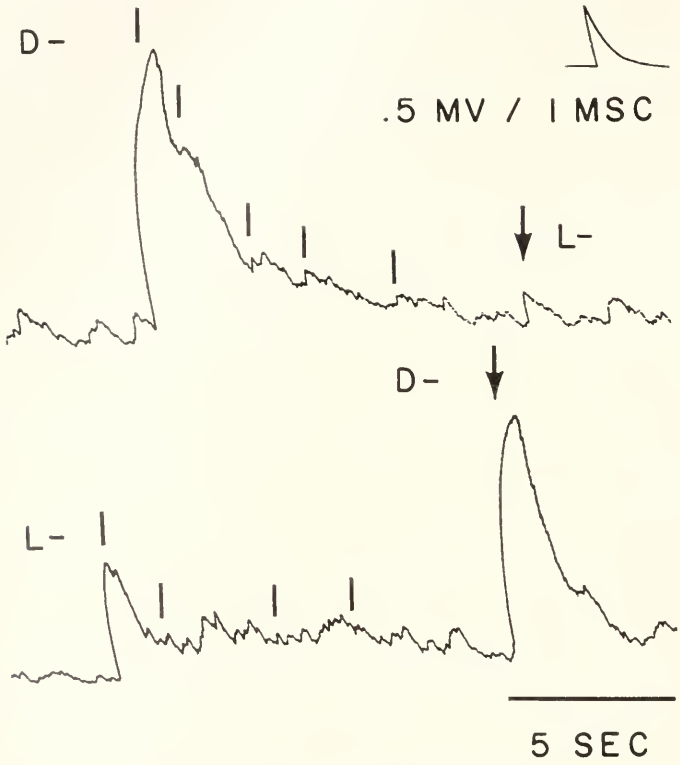


FIGURE 11. Demonstration that L-aspartic acid does not inhibit response to D-aspartic acid. Upper record, 5 drops 0.04 *M* D-aspartic acid, indicated by bars, followed without washing by 1 drop of 0.04 *M* L-aspartic acid, arrow. Lower record, 5 drops L-aspartic, at bars, followed without washing by 1 drop D-aspartic acid, at arrow. *C. antennarius.*

of aspartic acid the differential sensitivity may be attributed to a single receptor cell as shown in Figure 10, where the unit indicated with an arrow is obviously differentially responsive to both isomers. When one isomer is markedly less active than the other, prior application of the less active isomer appears to have no inhibitory effect on response to the more active isomer. An experimental record shown in Figure 11 illustrates this point: Application of five drops of D-aspartic acid almost completely prevents response to L-aspartic acid, suggesting that both isomers activate the receptor at identical sites. The largely undiminished response

to D-aspartic acid after five drops of L-aspartic acid suggests further that the latter does not obstruct these sites.

Discussion

These experiments confirm the electrophysiological demonstration by Case and Gwilliam (1961) of the presence of amino acid-sensitive receptors on the dactyls of crabs, and depict the two species, *Carcinus* and *Cancer*, as markedly alike in chemosensory specificities. Even though the earlier work by Case and Gwilliam did not encompass as many substances as the present investigation and was only roughly quantitative, similarities between the two organisms are still obvious. The dactyl receptors of neither are responsive to such organic acids as α-keto glutaric or glutaric, or to certain sugars and alcohols. L-glutamic acid is an extremely effective stimulus to both and is in both considerably more excitatory than its sterioisomer. Similar responses to the isomers of aspartic acid occur in both although the differential sensitivity to leucine isomers reported for *Carcinus* appears to be reversed in *Cancer*. Other discrepancies are evident, but are difficult to interpret owing to the differences in methods of evaluating responses in the two reports. Particularly unfortunate was the use of one of the most effective compounds, L-glutamic acid, as the standard in the work on *Carcinus* since it undoubtedly rendered less precise the evaluation of weakly effective compounds.

Laverack (1963) was unable to confirm our observations on the dactyl chemoreceptors of *Carcinus* (Case and Gwilliam, 1961) and described in that organism yet another category of chemoreceptor, characterized by long response latencies on the order of 10–15 *seconds*, slow adaptation and sensitivity to only a few amines. Since the present work on another species demonstrates receptors quite similar to those which we found in *Carcinus*, Laverack's negative observations require little comment other than to note that his manner of preparing the dactyl differed considerably from ours. Dissection of the dactyl, the method employed by Laverack, is likely to be considerably more damaging to the receptor innervation than our method of subdivision of the nerve trunk more proximally.

Laverack's observation of long latency units is most interesting and may well represent an unusual class of chemoreceptors. We have so far failed to demonstrate such units although the response to trimethylamine, which he found an effective stimulant, was distinctly prolonged in our preparations. But even so the response is far too early to fall within the latency class he describes.

The magnitude of the externally recorded action potentials in the long latency units, 400 μV in one instance, is surprisingly large, as Laverack remarks, especially when one reflects on the physiological incongruity of providing such sluggish receptors with rapidly conducting axons. The largest unmistakable chemoreceptor spikes which we have observed are in the neighborhood of 100 μV in the most optimal external recording situations, and the majority are 50 μV or less. Large spikes are readily observed upon mechanical stimulation of a dactyl hair sensillum, and this, in conjunction with the long latency of Laverack's large units to chemical stimulation but *not* to mechanical (see our Fig. 5, for example), suggests the possibility that Laverack might have observed mechanoreceptors activated nonspecifically by chemical stimuli. While the responses figured by Laverack (his Fig. 6) do show an early burst of impulses, evidently associated with stimulus application

and similar in spike size to the later chemical response, the data at the moment are insufficient to permit final determination of the problem.

The data of Table VI suggest that the dactyl receptors are not sensitive to peptides and proteins. While this seems quite plausible in the light of the sensitivity of the receptors to variation in structural details of the amino acids, it is also true that undoubtedly inappropriate proteins were utilized as test materials, owing to the problem of establishing purity. It must further be obvious that other receptor types may be present which do have sensitivity to peptides and proteins. Nevertheless, there appear to be no particular advantages for receptors mediating feeding responses to function as protein receptors as long as they are capable of detecting amino acids. Amino acids are considerably more soluble than proteins and hence are more likely to create a sensory trail to food sources. They lack the species specificity of proteins, which might introduce unwarranted specificity in the gustatory sense of the virtually omnivorous crabs.

While it is obviously unwise to argue from these observations that the arthropods in general do not possess protein receptors, there has been yet no proven instance of the electrophysiological demonstration of responsiveness to protein by an arthropod. Certainly the only other experiments known to us to be addressed to this question, those of Wallis (1961) on labellar chemoreceptor hairs of *Phormia*, in which haemoglobin and brain-heart infusion elicted neural activity, do not make a clear contribution to the problem. It is by no means obvious that these two substances, as applied, are amino acid-free, and they may not be free of other active contaminants, for example, the sugar and sodium chloride found in at least one commercial brain-heart infusion, which could be excitatory to the labellar receptors. There are, of course, compelling experiments in the behavioral literature which argue for the perception of host-specific, thermolabile materials by commensal organisms (Davenport, 1955). These may well be polypeptides or proteins.

Possible homology of neural junctions and sensory receptors (Grundfest, 1959) prompts inquiry into the similarities in the variety of amino acids to which they respond. Two classes of amino acid effects are evident at peripheral and central junctions: (1) excitatory, induced most strongly by dicarboxylic amino acids; and (2) inhibitory, principally due to the action of ω-amino acids. The dactyl chemoreceptors are responsive to many compounds from the first category and are not affected by ω-amino acids although certain compounds with junctional inhibitory effects are excitatory to them.

Robbins (1959) has reported excitation of crayfish muscle by L-glutamic acid at concentrations as low as 2×10^{-5} *M* with complete inactivity of D-glutamic acid. L-aspartic acid is somewhat less active than L-glutamic acid. The dactyl receptor threshold for L-glutamic acid is in the range of 10^{-5} *M* (Case and Gwilliam, 1961) and while D-glutamic acid is not without effect on the dactyl receptors, it is approximately three times less effective than the L-isomer. As in crayfish muscle, L-aspartic acid is considerably less effective than L-glutamic acid.

However, there is little correlation between inhibitory effectiveness of a compound on crayfish muscle and its excitatory effect on the dactyl receptors. Robbins (1959) lists in decreasing order of inhibitory effect on crayfish muscle: γ-aminobutyric acid > β-alanine = taurine > ϵ-aminocaproic acid. Taurine and β-alanine are highly effective dactyl receptor stimulants while the remaining mem-

bers of the series are without effect. The divergence thus indicated is emphasized by the reactions of the two preparations to DL-α-aminobutyric acid. The most effective excitant known for the dactyl receptors, it has neither excitatory nor inhibitory influence on crayfish muscle.

The extensive investigation by Curtis, Phillis and Watkins (1961) of amino acid effects on toad spinal neurons similarly indicates better correlation of dactyl receptor response with excitatory rather than with inhibitory compounds. Yet the correlations are by no means adequate enough to argue for more than the most general similarity in excitatory mechanisms between the two preparations. The great sensitivity of dactyl chemoreceptors to α-amino monocarboxylic acids, as well as to dicarboxylic acids, constitutes a major difference in the two preparations. Further divergence is seen in the insensitivity of toad spinal neurons to N-substitution in active compounds, this generally serving to impair activity in the dactyl preparation. Another dissimilarity lies in the generally greater excitatory effects of D- isomers on the toad neuron. The dactyl receptor may be differentially sensitive to either optical isomer or to neither.

As far as compounds inhibitory to toad spinal neurons are concerned, the correlation is as negative as between dactyl receptors and crayfish muscle. Taurine, β-alanine and γ-aminobutyric acid are all strongly inhibitory to the spinal neuron, while only γ-aminobutyric acid lacks an excitatory effect on dactyl receptors.

Finally, the possibility has been tested that junctional inhibitors, while lacking excitatory effects on the dactyl chemoreceptors, might reduce dactyl responses to effective stimulants (Case and Miller, unpublished observations). Pretreatment of dactyl receptors with γ-aminobutyric acid had no observable effect on responses to effective amino acids. The dactyl receptors are thus categorized as primarily responsive to α-amino acids with specificity sufficiently broad to include many junctional excitants but not the inhibitory ω-amino acids.

Summary

Chemoreceptors present on the distal limb segments of *Cancer antennarius* and *C. productus* have been examined by recording from axons dissected from the limb nerve. Receptor latencies are on the order of 35 msec., depending upon stimulant and concentration, and adaptation is rapid. Response intensity is linearly related to the logarithm of concentration. Optical isomers of certain compounds are discriminated and in one instance response is shown to be pH-dependent. Most effective stimulants are α-amino acids and related compounds. Among the most effective compounds are DL-α-amino-N-butyric acid, taurine, L-glutamic acid, and serine, in descending order of activities. Peptides are uniformly less active than their constituent amino acids, and two proteins were found to be without activity.

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